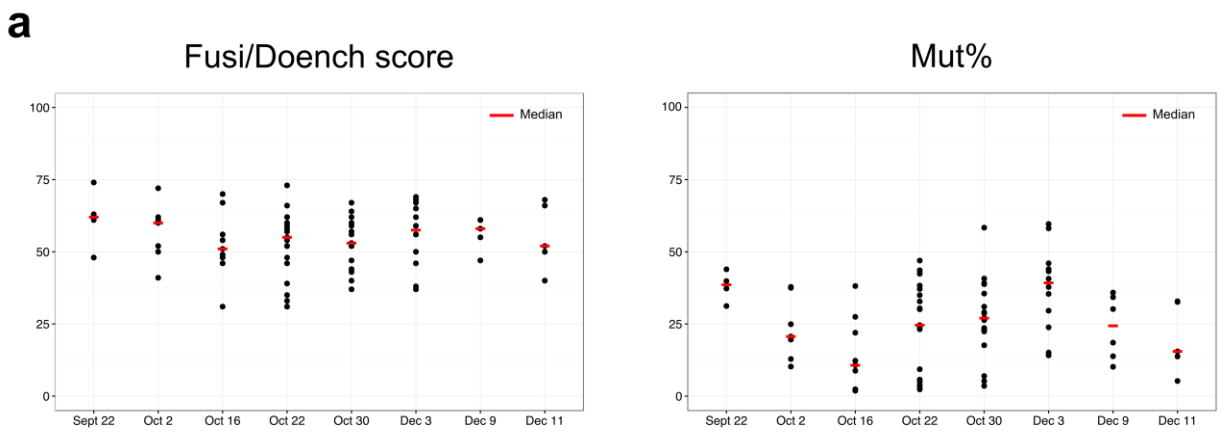
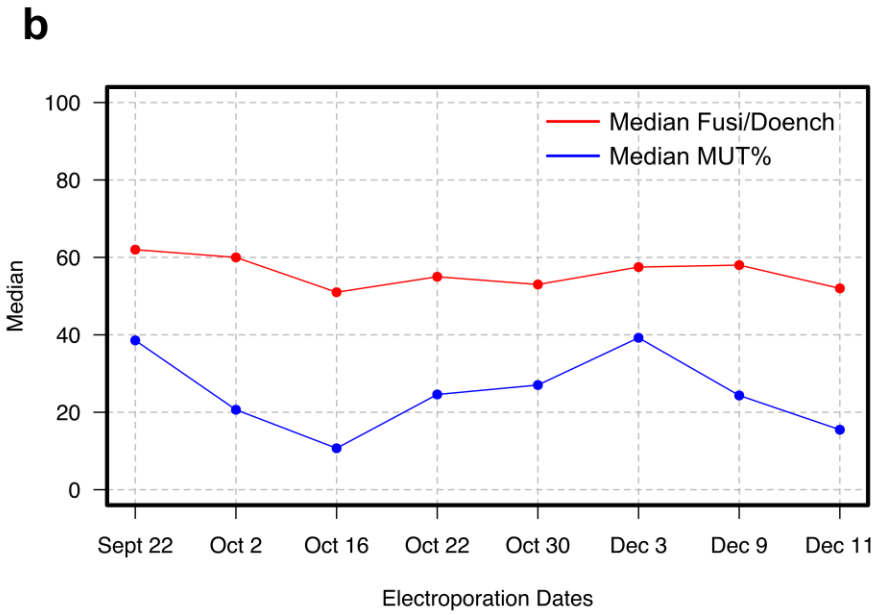
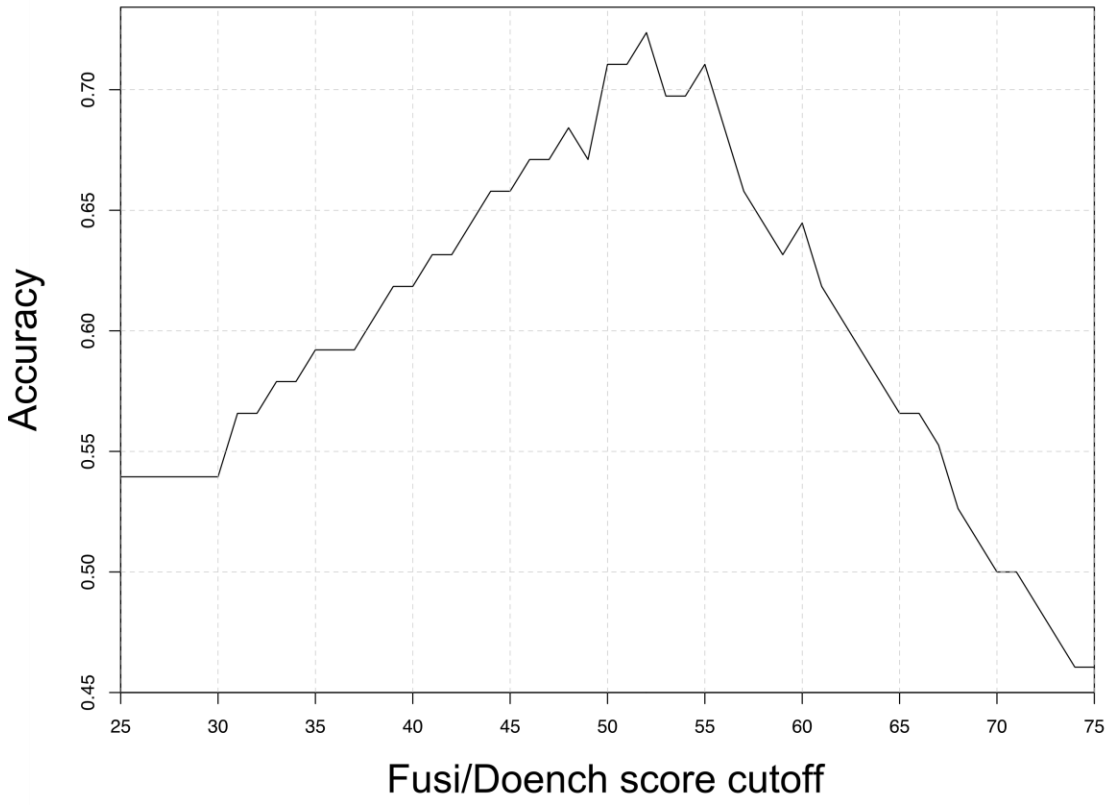




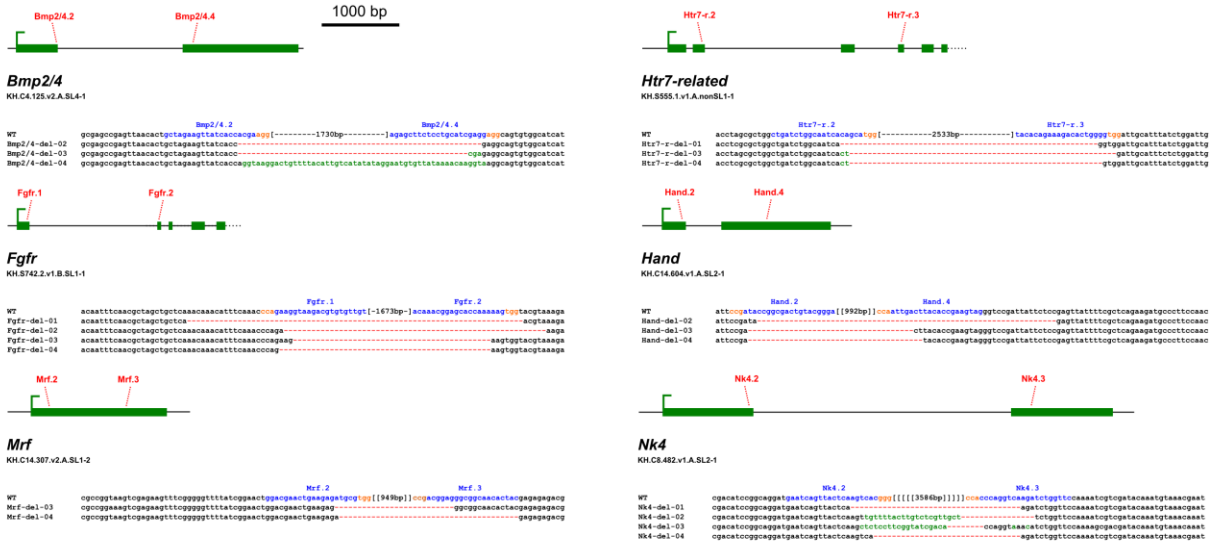
Supplementary Figure 1. Fusi/Doench scores and mutagenesis efficacies plotted by electroporation date

a) Fusi/Doench scores (left) and mutagenesis efficacy estimates ("Mut%", right) for individual sgRNAs tested, grouped by electroporation date. **b)** Plot of median values indicated in (a). While variation in Fusi/Doench score within and between dates should be random, mut% could in theory be affected by electroporation efficiency variation, or embryo batch effects. Despite the narrower range of Fusi/Doench scores, trends were similar for both datasets.



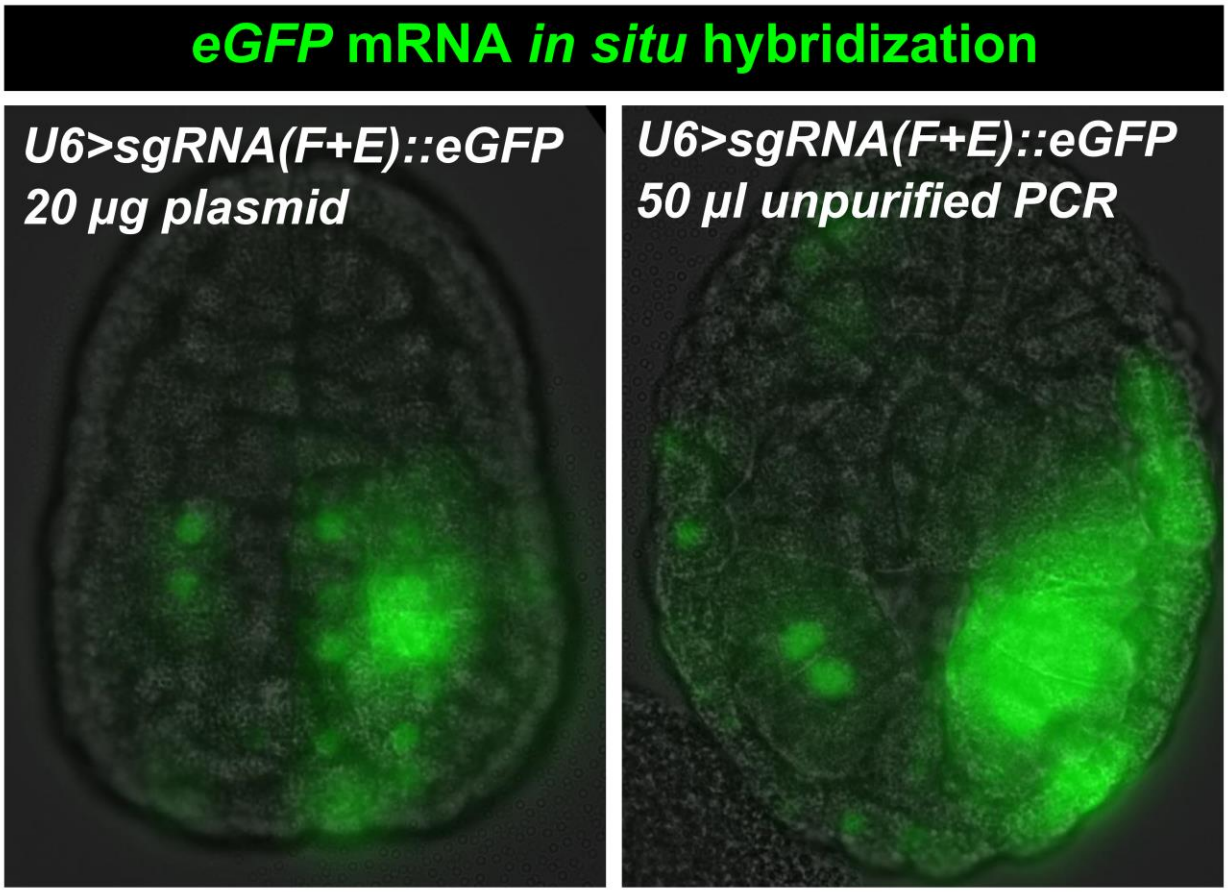
Supplementary Figure 2. Accuracy of good vs. bad sgRNA classification using different Fusi/Doench score cutoffs

Accuracy was defined as the percentage of correctly classified instances (True Positives + True Negatives)/(True Positives + True Negatives + False Positives + False Negatives). The maximum accuracy was 0.72, using a cutoff of 52. See **Supplementary Table 2** for data.



Supplementary Figure 3. Other examples of large deletions obtained by combinatorial action of two sgRNAs

Sequence alignments of clones for each locus, amplified from embryos in which two sgRNAs were used for CRISPR/Cas9-induced site mutagenesis.



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883 **Supplementary Figure 3. Evidence of *in vivo* transcription from electroporated, unpurified PCR**

884 **products**

885 *In situ* hybridization of *eGFP* in late gastrula/early neural stage embryos electroporated with either *U6>*

886 *sgRNA(F+E)::eGFP* plasmid (20 µg) or unpurified PCR product (50 µl, ~5 µg DNA).

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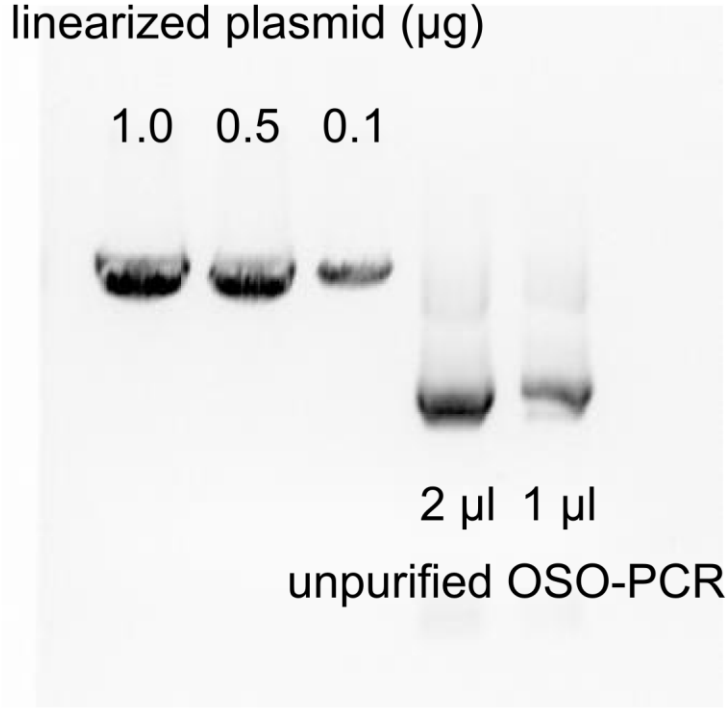
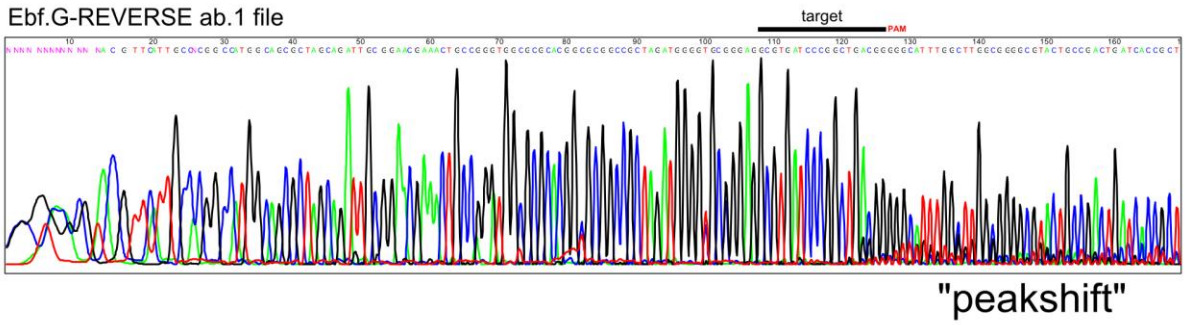


Supplementary Figure 5. Quantification of OSO-PCR products

Image of gel electrophoresis of varying amounts of linearized plasmid and unpurified OSO-PCR products. Pixel intensity analysis in ImageJ was performed as previously described (STOLFI *et al.* 2014), and indicated that the sgRNA expression cassette in unpurified OSO-PCR reactions are at a concentration of approximately 100 ng/µl.



Supplementary Figure 6. Detection of CRISPR/Cas9-induced indels by Sanger sequencing

Chromatogram of *Ebf* amplicon from embryos targeted using the Ebf.G sgRNA. "Peakshift" shows superimposed sequence peaks as a result of the resulting mix of mutant alleles bearing short indels around the Ebf.G target site and PAM. This peakshift can be quantified and corrected to produce a precise quantification of CRISPR/Cas9-mediated mutagenesis (see **Materials and methods** for details).